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Toxoplasma Antigens Recognized by Immunoglobulin M and G Antibodies during Acute and Chronic Infection in Humans

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Abstract

Toxoplasma antigens recognized by immunoglobulin M (IgM) and IgG antibodies in sera of Japanese patients with acute and chronic infection were analyzed by using immunoblotting technique. The both IgM and IgG antibodies in sera of chronic patients mainly recognized the antigenic components with molecular weights (m.w.) of $\geq$35,000 although the most of the bands observed in the IgM blots were faint. The IgM and IgG antibodies in sera of acute patients recognized the antigenic components with a wide m.w. range of $\geq$6,000. The antigens with m.w. of 30,000, 22,000 and 6,000 in the IgM blots and those with m.w. of 42,000, 41,000, 30,000, 27,000 and 6,000 in the IgG blots were strongly recognized by the antibodies of only acute patients. Those antigens appear to be useful for serodiagnosis to distinguishing the acute from chronic stages of infection.

Key words: *Toxoplasma* infection, antigens, IgM antibody, IgG antibody, immunoblotting, serodiagnosis

Introduction

The most commonly recognized clinical manifestation of acute acquired infection with *Toxoplasma gondii* in immunocompetent adults is lymphadenopathy (Krick and Remington, 1978). Although criteria for histopathologic diagnosis of toxoplasmic lymphadenopathy have been described (Dorfman and Remington, 1973), serologic diagnosis is preferable to having the patients undergo a biopsy (Brooks *et al.*, 1987; Kobayashi *et al.*, 1983). However, the high prevalence of toxoplasma antibodies in otherwise normal individuals because of chronic (latent) infection and the fact that titers may remain elevated for years following the acute infection have complicated interpretation of serologic test results obtained in individuals suspected of having acute toxoplasmosis.

Recently, we revealed that use of the antigens which are present in tachyzoites but not in bradyzoites (tachyzoite-specific antigens) are important in serodiagnosis for distinguishing the acute from chronic stages of infection (Suzuki *et al.*, 1988, 1990). The tachyzoite-specific antigens were recognized by IgG antibodies which were present in sera of patients only in the acute stage of infection (Suzuki *et al.*, 1988, 1990). Immunoblotting is useful technique for analyzing antigens recognized by antibodies in sera of patients. However, few reports have been published regarding *Toxoplasma* antigens recognized by IgM and IgG antibodies in Japanese patients. In order to develop sensitive and specific serologic tests for diagnosis of acute toxoplasmosis in Japanese patients, it is essential to analyze antigenic components recognized by the antibodies of Japanese patients. In the present study, we analyzed antigenic components of *T. gondii* which were recognized by IgM and IgG antibodies in sera of Japanese patients with either acute or chronic infection by using immunoblotting technique. We revealed a presence of antigenic components recognized by the antibodies during only the acute stage in those patients.

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Materials and Methods

Human sera. Sera were from 5 Japanese

patients who had acute lymphadenopathic toxoplasmosis on 1 to 6 months after onset of lymphadenopathy. Diagnosis of acute lymphadenopathic toxoplasmosis was by both serology and histopathology (Kobayashi *et al.*, 1983; Welch *et al.*, 1980). Control sera were from 10 chronically infected Japanese individuals who were healthy and had known stable toxoplasma IgG antibody titers (latex agglutination test titers ranged from 1:64 to 1:1,024) for more than one year. Sera were also obtained from 17 healthy seronegative individuals.

Mice. Outbred female ddY mice (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu) were 6 weeks old when used.

Toxoplasma antigens. Tachyzoites of the RH strain were harvested from peritoneal fluids of mice infected 3 days earlier and purified as described previously (Wilson *et al.*, 1980). Fresh tachyzoites were solubilized in the sample buffer described by Laemmli (1970).

Gel electrophoresis and immunoblotting. Electrophoresis was performed in 13% polyacrylamide slab gels with the discontinuous sodium dodecyl sulfate buffer system described by Laemmli (1970). Molecular weight standards were lysozyme, β-lactoglobulin, trypsinogen, pepsin, egg albumin, and bovine serum albumin (Sigma, St. Louis, U.S.A.). For immunoblots, proteins separated by electrophoresis were transferred to nitrocellulose paper as described by Towbin *et al.* (1979). Blots were first soaked in 5% nonfat milk in phosphate-buffered saline (PBS; pH7.2) and then incubated with patients sera at a 1/100 dilution as described previously (Suzuki *et al.*, 1988). Thereafter, the nitrocellulose sheets were incubated with horseradish peroxidase-conjugated rabbit anti-human IgM and IgG antibodies (Cappel, West Chester, PA, U.S.A.) at a dilution previously determined to be optimum. The color development was with a solution containing 0.1 mg of diaminobenzidine per ml of 0.1% H_2O_2 in PBS (Suzuki *et al.*, 1988).

Results

Toxoplasma antigens recognized by IgM antibodies in sera of patients with chronic infection. Sera from 10 individuals with chronic (latent) infection were applied to immunoblotting to analyze *Toxoplasma* antigens recognized by IgM antibodies in those sera. The blots reacted with each of those sera showed numbers of bands but all of them were faint (Fig. 1). Those bands were mainly observed in a range of m.w. of $\geq$35,000. Four antigens with m.w. of 70,000, 51,000, 39,000 and 35,000 were most frequently recognized by IgM antibodies among the chronic patients (arrowed in Fig. 1).

Toxoplasma antigens recognized by IgG antibodies in sera of patients with chronic infection. Sera from the chronic patients showed numbers of clear bands in the IgG blots in contrast to the IgM blots (Fig. 1). The antigens recognized by the IgG antibodies were mainly in a range of m.w. of $\geq$35,000 as same as observed in the IgM blots. Six antigens with m.w. of 82,000, 70,000, 62,000, 51,000, 39,000 and 35,000 were the major bands in the IgG blots (arrowed in Fig. 1). Those antigens were commonly recognized by the antibodies of all of the chronic patients tested although there were variations in recognition of antigens between the individuals.

Toxoplasma antigens recognized by IgM antibodies in sera of patients with acute infection. Sera obtained from 5 patients with acute infection were applied in immunoblotting. IgM blots reacted with sera from those patients represented 3 to 8 apparent bands (Fig. 2). The antigens with m.w. of 39,000 and 6,000 were commonly recognized by IgM antibodies of all of the 5 patients (arrowed in Fig. 2) although there were variations in recognition of the other antigens among the individuals. The antigens with m.w. of 30,000 and 22,000 were strongly recognized by the antibodies from 2 of the 5 patients (arrowed in Fig. 2).

Toxoplasma antigens recognized by IgG antibodies in sera of patients with acute infection. The IgG blots reacted with acute patients sera represented many clear bands in a wider range of m.w. than the blots reacted with chronic

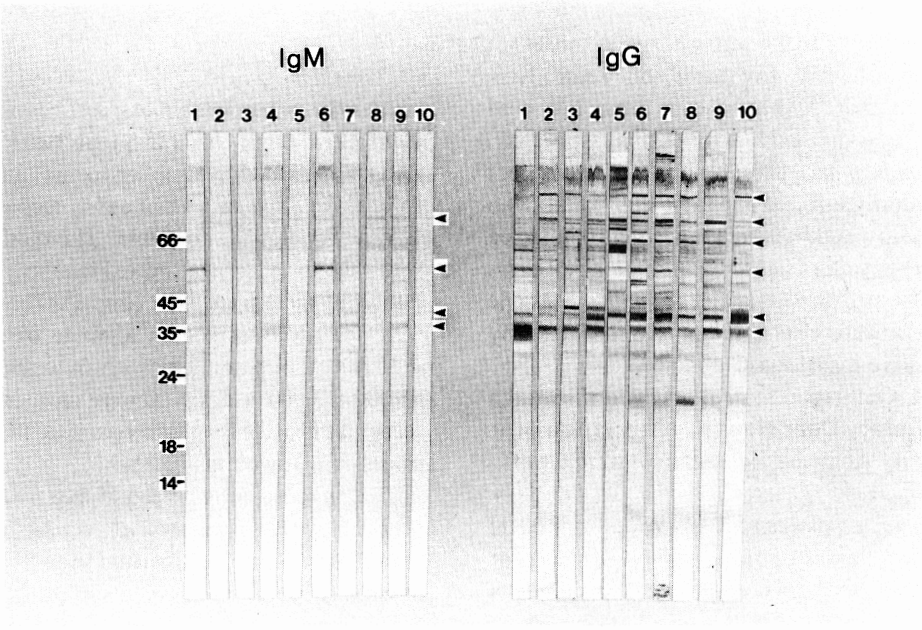

Fig. 1 Immunoblot analysis of *T. gondii* antigens recognized by IgM and IgG antibodies in sera of patients with chronic infection. Numbers on the left represent m.w. standards. Arrows indicate the major antigens commonly recognized by the antibodies of each of those patients. The both IgM and IgG blots of each patient are represented in the same number.

patients sera (Fig. 2). The six antigenic components in a range of m.w. of $\geq$35,000 which were recognized by IgG antibodies of chronic patients (arrowed in Fig. 1) were also recognized by the antibodies of acute patients (Fig. 2). In addition to those antigens, the antibodies of the acute patients strongly recognized 3 to 9 antigenic components in a range of m.w. of 6,000 to 30,000. The antigens with m.w. of 42,000, 41,000, 30,000, 27,000, and 6,000 (arrowed in Fig. 2) were clearly recognized by IgG antibodies of all of the 5 acute patients but not by those of chronic patients in Fig. 1. In contrast to those antigens, the antigenic components whose m.w. were 70,000 and 35,000 were more strongly recognized by IgG antibodies of chronic patients (Fig. 1) than those of acute patients (Fig. 2).

Toxoplasma antigens recognized by IgM and IgG antibodies in sera of seronegative individuals. Sera from 17 seronegative individuals were applied in immunoblotting. The both IgM and IgG blots reacted with each of those sera showed some of faint bands. The antigens with m.w. of 49,000 and 35,000 and those with m.w. of 82,000, 49,000 and 35,000 were most frequently recognized by IgM and IgG antibodies of those individuals, respectively.

Discussion

The results described above reveal that the both IgM and IgG antibodies of Japanese patients with acute infection recognized the different antigens of *T. gondii* from those of the patients with chronic (latent) infection. The major difference was observed in recognition of antigens in a range of m.w. of $\leq$30,000 in the both IgM and IgG antibodies. The antigens in that range were mainly recognized by the antibodies of only acute patients. Natural antibodies present in sera of seronegative individuals did not recognize those antigens in a range of m.w. of $\leq$30,000.

Regarding IgM antibodies, the antigenic components with m.w. of 30,000, 22,000 and 6,000

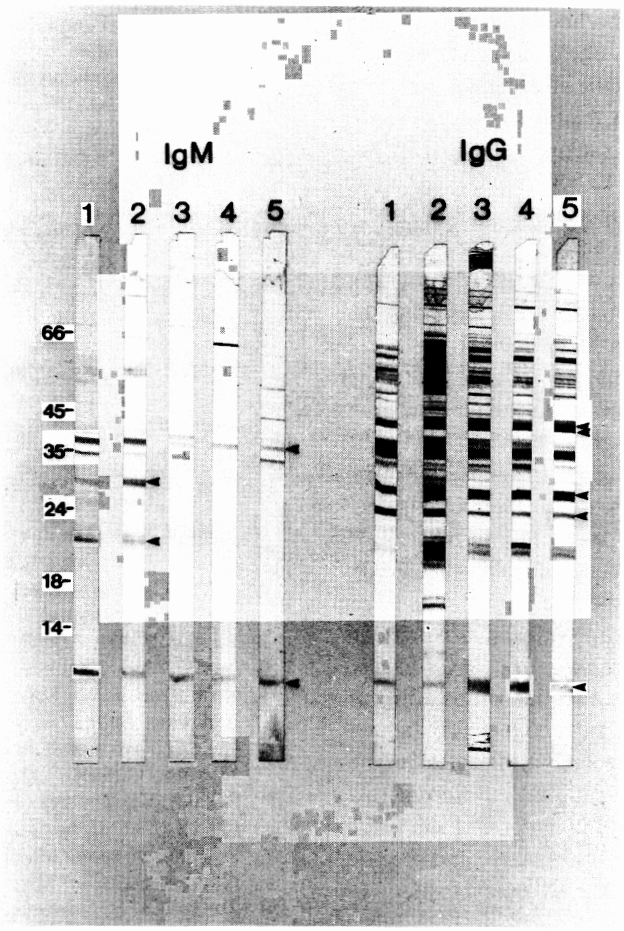

Fig. 2 Immunoblot analysis of *T. gondii* antigens recognized by IgM and IgG antibodies in sera of patients with acute infection. Numbers on the left represent m.w. standards. Arrows indicate the antigens strongly recognized by the antibodies of the acute patients but not by those of the chronic patients in Fig. 1. The both IgM and IgG blots of each patient are represented in the same number.

were the molecules strongly recognized by the antibodies of only acute patients. This fact strongly suggests that use of those antigens for detection of IgM antibodies would be very useful for serodiagnosis of acute toxoplasmosis in Japanese patients. It was reported by the researchers in the United States (Sharma *et al.*, 1983) and those in Finland (Partanen *et al.*, 1983) that antigenic components with m.w. of 32,000, 22,000 and 6,000 (Sharma *et al.*, 1983) or those with m.w. of 35,000, 25,000 and 6,000 (Partanen *et al.*, 1983) were the major antigens recognized by IgM antibodies of the acute patients. It is not clear whether the antigen component with m.w. of 30,000 in the present study is identical or not to those with m.w. of 35,000 or 32,000 in the other reports.

Regarding IgG antibodies, the antigenic components with m.w. of 42,000, 41,000, 30,000, 27,000, and 6,000 were recognized by the antibodies of only the acute patients. We previously reported that tachyzoite-specific antigens were

recognized by IgG antibodies which were present only during the acute stage of infection and use of those tachyzoite-specific antigens for detection of IgG antibodies was valuable for serodiagnosis of acute toxoplasmosis (Suzuki *et al.*, 1988). The antigens with m.w. of 42,000, 30,000 and 6,000 in the present study appear to be identical with the tachyzoite-specific antigens with the same m.w. in the previous study (Suzuki *et al.*, 1988). The other antigens which were revealed to be recognized by the antibodies of only acute patients in the present study might also be tachyzoite-specific antigens although they are not analyzed yet. We recently purified some of tachyzoite-specific antigens (m.w. 52,000 and 6,000) and applied those antigens to an enzyme-linked immunosorbent assay test to detect IgG antibodies in sera of patients (Suzuki *et al.*, 1990). That test was highly specific and sensitive for diagnosis of acute toxoplasmosis in American and French patients (Suzuki *et al.*, 1990). In case of Japanese patients, purification of the antigens which were revealed to be recognized by acute-stage-specific IgM and IgG antibodies in the present study and application of those purified antigens for detection of the antibodies will make possible to develop a highly sensitive and specific test for diagnosis of acute toxoplasmosis.

Acknowledgements

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References

1) Brooks, R. G., McCabe, R. E. and Remington, J. S. (1987): Role of serology in the diagnosis of toxoplasmic lymphadenopathy. Rev. Infect. Dis., 9, 1055–1062.
2) Dorfman, R. F. and Remington, J. S. (1973): Value of lymphnode biopsy in the diagnosis of acute acquired toxoplasmosis. N. Engl. J. Med., 289, 878–881.
3) Kobayashi, A., Watanabe, N., Suzuki, Y., Makioka, A., Katakura, K., Hamada, A. and Hirai, N. (1984): Serologic diagnosis of toxoplasmic lymphadenitis. Jpn. J. Parasitol., 33, 369–376.
4) Krick, J. A. and Remington, J. S. (1978): Toxoplasmosis in the adult – an overview. N. Engl. J. Med., 298, 550–553.
5) Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bactriophage T4. Nature (London), 227, 680–685.
6) Partanen, P., Turunen, H., Paasivuo, R. T. and Leinikki, P. O. (1983): Immunoblot analysis of Toxoplasma antigens by human immunoglobulin G, M, and A antibodies at different stages of infection. J. Clin. Microbiol., 20, 133–135.
7) Sharma, S. D., Mullenax, J., Araujo, F. G., Erlich, H. A. and Remington, J. S. (1983): Western blot analysis of the antigens of *Toxoplasma gondii* recognized by human IgM and IgG antibodies. J. Immunol., 131, 977–983.
8) Suzuki, Y., Thulliez, P., Desmonts, G. and Remington, J. S. (1988): Antigen(s) responsible for immunoglobulin G responses specific for the acute stage of *Toxoplasma* infection in humans. J. Clin. Microbiol., 26, 901–905.
9) Suzuki, Y., Thulliez, P. and Remington, J. S. (1990): Use of acute-stage-specific antigens of *Toxoplasma gondii* for serodiagnosis of acute toxoplasmosis. J. Clin. Microbiol., 28, 1734–1738.
10) Towbin, H. T., Staehelin, T. and Gordon, J. (1979): Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA, 76, 4350–4354.
11) Welch, P. C., Masur, H., Jones, T. C. and Remington, J. S. (1980): Serodiagnosis of acute lymphadenopathic toxoplasmosis. J. Infect. Dis., 142, 256–264.
12) Wilson, C. B., Tsai, V. and Remington, J. S. (1980): Failure to trigger the oxidative metabolic bursts by normal macrophages: possible mechanism for survival of intracellular pathogens. J. Exp. Med., 151, 328–346.